Journal of Plant Nutrition
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597277

Nitrogen Fertilizer Influences Fruit Quality, Soil Nutrients and Cover Crops, Leaf Color and Nitrogen Content, Biennial Bearing and Cold Hardiness of 'Golden Delicious'

John Thomas Raese [a]; Stephen R. Drake [a]; Eric A. Curry [a]
[a] United States Department of Agriculture, Agricultural Research Service Tree Fruit Research Laboratory, Wenatchee, Washington, USA

Online Publication Date: 01 October 2007
To cite this Article: Raese, John Thomas, Drake, Stephen R. and Curry, Eric A. (2007) 'Nitrogen Fertilizer Influences Fruit Quality, Soil Nutrients and Cover Crops, Leaf Color and Nitrogen Content, Biennial Bearing and Cold Hardiness of 'Golden Delicious'', Journal of Plant Nutrition, 30:10, 1585 - 1604
To link to this article: DOI: 10.1080/01904160701615483
URL: http://dx.doi.org/10.1080/01904160701615483

Journal of Plant Nutrition, 30: 1585–1604, 2007

ISSN: 0190-4167 print / 1532-4087 online
DOI: 10.1080/01904160701615483

Nitrogen Fertilizer Influences Fruit Quality, Soil Nutrients and Cover Crops, Leaf Color and Nitrogen Content, Biennial Bearing and Cold Hardiness of 'Golden Delicious'

John Thomas Raese, Stephen R. Drake, and Eric A. Curry

United States Department of Agriculture, Agricultural Research Service Tree Fruit Research Laboratory, Wenatchee, Washington, USA

ABSTRACT

Four rates of ammonium nitrate (NH_4NO_3) (0, 151, 454, and 908 g actual N/tree) were applied each spring for 6 years to 'Golden Delicious' (*Malus domestica*) apple trees. High rates of nitrogen (N) increased N concentration of Orchardgrass (*Dactylis glomerata)* blades and increased cover-grass growth whereas various legume species were prevalent at the low rates. Leaf N in spur or mid-terminal leaves increased yearly, and was related to leaf color by visual comparison and reflectance. Fruit from the higher N rates had greener peel and lower firmness, soluble solids content and titratable acidity. *In vitro* freeze tests indicated trees fertilized with lower rates of N were more cold hardy during the fall, winter and spring than those receiving the higher rates. In a similar long-term study on 'Delicious,' cold hardiness was related not only to seasonal temperature cycles and shoot dry matter, but to total sugars and sorbitol content in wood or sap.

Keywords: quality, fruit color, thinning, growth, vigor

INTRODUCTION

Nitrogen (N) containing fertilizers have been commonly applied to fruit trees in the Pacific Northwest over the past few decades to increase vigor and yield (Batjer et al., 1966). Furthermore, N is a principle factor that influences leaf and fruit color of 'Golden Delicious' apple trees (Bensen et al., 1957; Williams and

Received 27 March 2006; accepted 23 October 2006.
Address correspondence E. A. Curry, USDA, ARS, TFRL, 1104 N. Western Avenue, Wenatchee, WA 98801. E-mail: Curry@tfrl.ars.usda.gov

Billingsley, 1974; Yamazaki et al., 1977). The use of color charts to predict the N concentration in apple leaves of 'McIntosh,' 'Stayman' and 'York' have been proposed earlier (Compton et al., 1946; Shear and Horsfall, 1948). This study of the apple (*Malus domestica*) cultivars, 'Delicious' and 'Golden Delicious,' shows important tree responses to all seasons of the year for ten and 6 year periods, respectively. The authors are not aware of previous reports of such comprehensive observations on these cultivars throughout the seasons.

In the 'Golden Delicious' experiment nitrogen (N) fertilizer was applied at one of four rates each spring for six years. The objectives were to determine the effects of the N broadcast treatments on soil analysis, cover crop growth and N analysis, tree growth and fruit yield as it influences biennial bearing, prediction of leaf N concentrations and fruit color by subjective and objective measurements of leaf color and determination of fruit quality by not only N treatments but time of harvest and location of fruit on the tree. Additional objectives were to determine 1) the influence of N treatments on cold hardiness throughout fall, winter and spring and 2) if other measured components might be implicated as markers of cold hardiness.

In the 'Delicious' study, samples of 1- and 2-year wood were collected at nearly weekly intervals throughout the year over a 10-year period to determine cold hardiness and other factors that may be involved in cold hardiness such as content of various sugars, dry matter and sap in 2-year wood. This latter study was included to assist in explaining the possible causes for cold hardiness that were observed in the 'Golden Delicious' experiment.

Winter injury to fruit trees can be a major concern some years as previously reported (Ketchie et al., 1976) in north central Washington state where recorded low temperatures ($-38°C$), in the colder areas, caused severe injury to numerous fruit orchards in 1968.

MATERIALS AND METHODS

'Golden Delicious' Experiments

Treatments and Experimental Design

Fully mature 'Golden Delicious' apple trees on seedling rootstock were grouped into four-tree subplots according to slope location in approximately a seven hectare area until 11 such subplots (replicates) were obtained. In each of the four-tree sub-plots, 0, 151, 454, and 908 g of actual N per tree were assigned resulting in 4 N treatments and 11 replications for a total of 44 trees in the experiment. The trees were spaced 8×8 meters apart and inter-spaced with 'Winesap' apple trees that provided adequate distance between trees to minimize nutrient contamination. The N fertilizer was ammonium nitrate (NH_4NO_3) broadcast by hand each March for six years under the drip line of the tree. Location of the orchard was at the Washington State University Experiment Station, south of Orondo, WA. Statistical analysis was determined by MSTAT-C. Based on significant F-testing, mean separation analysis was determined by Duncan's Multiple Range Test ($P \leq 0.05$).

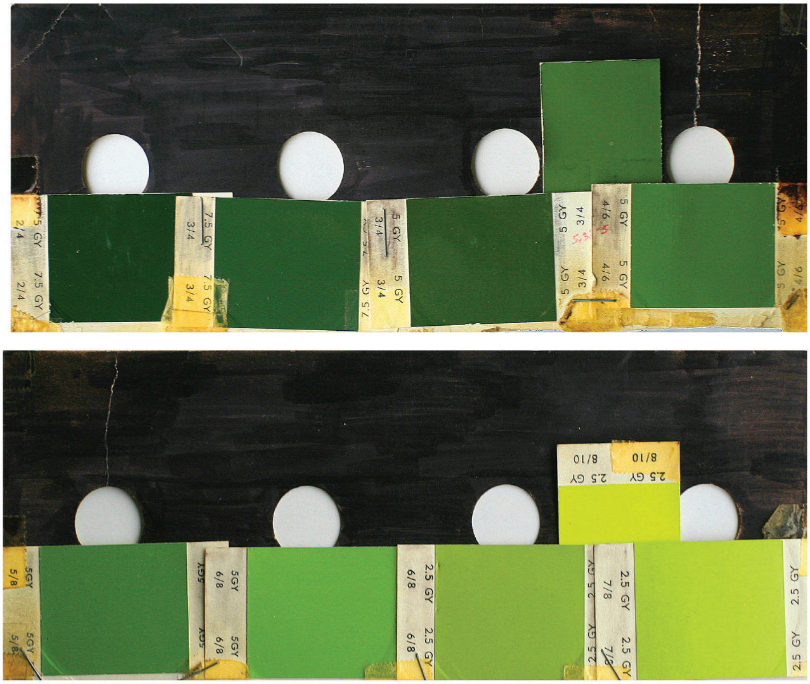
Soil analyses and cover crops: Trees were grown in a Burch loam with a 3 to 8% westerly slope. Soil samples were collected at 15 to 20 cm depths under the branches of each tree and sent to the Soil Analysis Dept., Washington State University, Pullman, WA. Sampling occurred in the summer during the early part of the experiment and at the next to last year of the study. The cover crop consisted primarily of orchardgrass (*Dactylis glomerata)*, but legumes including alfalfa (*Medicago sativa*), red clover (*Trifolium pratense*), and white clover (*Trifolium repens*) were present in the low N plots. The above cover crop species were visually estimated for percentage of cover species. Twenty orchardgrass blades were measured around each tree for height and blade width and samples were analyzed for N content (Lindner and Harley, 1942). Blade color was estimated by comparison to Munsell color chips (Munsell, 1971).

Apple Leaf N Concentrations, Fruit Color and Leaf Color Estimates

Samples of spur and mid-terminal leaves, 20 each, were collected from mid-May to October for N analysis and leaf color estimates. Nitrogen was determined from 1-cm leaf discs by the Nessler method (Lindner and Harley, 1942) and modified for use with selenium oxychloride in place of hydrogen peroxide. Leaf color was determined by visually comparing the upper leaf surface with Munsell color chips and designated with numerical values of hue, value and chroma (Munsell, 1971). Leaf color was also objectively determined with a Bausch and Lomb Spectronic 20 colorimeter by measuring the percent reflectance of the upper leaf surface at 558 um. Fruit color from 20 apples was estimated by visually comparing apple peel color at harvest with shades of color on the USDA standard ground color chart for apples and pears in the western states (issued July 1929). A similar hand-held color chart with 10 Munsell color chips and holes 2.0 cm in diameter and with a black background was improvised by the senior author for leaf color estimates (Figure 1).

Bloom, Yield, and Tree Growth

Each spring, bud stage development was estimated visually where 2 = green tip and 8 = full bloom of apple trees (Washington State University, 1981). Percentage of return bloom was visually estimated by two or three persons each spring, as well as yield in terms of 19 kg boxes (counted 100 fruit/box) per tree before fruit thinning and at harvest. Trunk circumference was measured in April each year. Shoot extension was measured from 20 shoots/tree throughout the growing season and leaf weight was determined by measuring fresh weight of a 20-leaf sample.



Hue	Value	Chroma	Reflectance (%)	Nitrogen (%)
7.5 gy[z]	2.5	4.0	12.9	2.47
7.5 gy	3.0	4.0	14.1	2.24
5.0 gy	3.0	4.0	14.4	2.24
5.0 gy	3.5	5.0	18.5	1.99
5.0 gy	4.0	6.0	22.9	1.84
5.0 gy	5.0	8.0	27.5	1.53
5.0 gy	5.0	8.0	33.8	1.48
2.5 gy	6.0	8.0	38.0	1.39
2.5 gy	7.0	8.0	42.9	1.21
2.5 gy	8.0	10.0	55.0	1.05

[z]gy = green/yellow

Figure 1. Chart for comparing color of 'Golden Delicious' spur and mid-terminal apple leaves using Munsell color chips (Munsell, 1971) designated with numerical values of hue, value and chroma to predict leaf N concentration (Raese, 1978; Raese and Williams, 1974). The above leaf color designations and N concentrations were derived from samples obtained monthly, July through October, from 20 leaves per treatment replication during the six-year study.

Fruit Quality

Fruit color from 20 apples was measured visually as noted earlier with the USDA color chart and determined electronically with a Color Difference Meter and an Agtron model E5-W. Fruit size was determined by weighing, and measuring the circumference of each apple. Fruit firmness was determined with a Magness-Taylor firmness tester equipped with an 11.1 cm. tip, at 3 locations on each apple after peel removal. Soluble solids concentration (SSC) was determined with a Bausch and Lomb refractometer as a composite sample from extracted juice. Titratable acidity (TA) was determined as previously described. Values from the above samples represent the mean of 20 apples from each replication of the four N fertilizer treatments, three harvest times, and fruit from the upper or lower part of the tree.

Cold Hardiness

Cold hardiness of 1- and 2-year wood and spurs was determined throughout the seasons by gradually freezing the samples at appropriate seasonal temperatures in freeze cabinets and then measuring the electrolytes using a conductivity meter as described previously (Ketchie et al., 1972; Raese, 1996; Raese et al., 1977, 1978) with minor modifications. Samples of wood from each treatment weighing 4 g were cut into 1 cm pieces, placed into 50 mL beakers and frozen in a Tenny Freeze Cabinet with a Leeds and Northrup programmer at the rate of 2.8°C per h drop from 0°C until reaching one of three freeze temperatures (low, moderate or high at 5°C apart) appropriate for the season. After a period of thawing (2 h) at 21°C, 20 mL of deionized water were added to the beaker. Electrolyte leakage from the wood samples was determined after 24 h. Subsequently, to determine percent electrolyte leakage, the samples were boiled for 7 min. and, after returning liquid to original volume with deionized water, conductivity was again measured after 24 h for total electrolytes. Levitt (1956) described the often-used T_{50} as the temperature at which 50% of the tissue was damaged. Cold hardiness (T_{50}) was determined by graphing total electrolytes obtained from the three freeze tests against their temperature and $T_{50}$was determined at the intercept of the constant (42.5) electrolytes and the resulting temperature (Ketchie et al., 1972).

‘Delicious’ Experiment

In an orchard adjacent to that used for experiments on ‘Golden Delicious’ were fully mature ‘Red King Delicious’ apple trees grown in a Cashmont cobbly sandy loam soil with a westerly slope of 3 to 15%. Over a period of ten years, samples of 1, 2, and 3 year-old wood were obtained at weekly intervals to support and closely supplement the ‘Golden Delicious’ experiment. Samples of 1- and 2-year old wood were collected throughout each year from the same five apple trees. Cold hardiness was determined for 'Delicious' apple trees in the same manner as previously described for 'Golden Delicious' apple trees.

Sap was extracted under vacuum from 2-year old shoots 30-36 cm in length by cutting 12.7 mm length sections at 5 second intervals (Williams and Raese, 1974). Sorbitol, fructose, glucose and sucrose were determined in ground, dried 2-year old wood and extracted at 60°C with 80% ethanol for 3 h in a soxhlet apparatus (Williams and Raese, 1974). Sorbitol in sap and sorbitol and sugar tri-methylsulfite derivatives were detected by gas chromatography (Williams and Martin, 1967).

RESULTS AND DISCUSSION

'Golden Delicious' Experiments

Soil Analysis

As the experiment progressed over time, soil analyses indicated that soil pH was reduced by higher rates (454 and 908 g) of N per tree (Table 1). In an earlier report (Raese, 1995), soil pH was reduced by ammonium nitrate versus calcium nitrate at high rates of N fertilizer and red peel color of 'Delicious' was related to higher soil pH values and lower concentrations of manganese (Mn) in fruit peel. In the current study, organic matter, boron (B), and zinc (Zn) levels in the soil were slightly higher with the high N rates than the no N treatment. Soil nitrate-N (NO_3-N) was considerably higher with the high rate of N. Soil phosphorus (P), magnesium (Mg) and salts were lower with the higher N rates. It is particularly important to note that soil calcium (Ca) levels were lowest with the high rate of N. This finding of lower soil Ca and pH levels are perhaps related to the higher incidences of bitter pit associated with high N fertilizer of apple trees (Raese, 1995; 2000). In this study, potassium (K) levels in the soil were not affected by N rates.

Cover Crop

In the orchardgrass cover crop, blade N concentration, height and width were markedly increased by increasing rates of N (Table 2). The proportion of orchardgrass to legumes (white clover, red clover and alfalfa) was also higher with N applications versus no N. Visual comparisons of blade color of orchardgrass with Munsell color chips were significantly related to N treatment rates. This indicates that a considerable amount of N fertilizer ultimately appears to go into producing grass rather than tree growth. However, in an indirect way, the cover crop may not only serve to prevent soil erosion, but also it could improve fruit quality in the long run, because the grass cover crop is utilizing the excess N.

Table 1
Effect of rate of nitrogen (N) fertilizer on soil properties after six years of nitrogen treatment of 'Golden Delicious' apples trees

Treatment	Soil	Organic	P	K	B	Zn	NO_3-N	Ca	Mg	Salts
N (g/tree)	pH	Matter (%)	(ppm)					(Meq/100 g)		(μmhos/cm)
0	6.80a[z]	2.06b	15.4a	365a	0.47b	11.0b	1.00b	9.3a	2.73a	0.248a
151	6.76a	2.21a	15.9a	378a	0.58a	13.0a	1.09b	9.7a	2.74a	0.240ab
454	6.62b	2.22a	13.3c	362a	0.54ab	12.0ab	1.24b	10.0a	2.66a	0.233bc
908	6.15c	2.18a	14.3b	352a	0.51ab	14.0a	2.20a	7.9b	2.15b	0.225c

[z]Means in a column not followed by a common letter are significantly different by Duncan's Multiple Range Test ($P \leq 0.05$).

Table 2
Effect of rate of nitrogen (N) fertilizer on properties of orchardgrass cover crop under 'Golden Delicious' apple trees

Treatment N (g/tree)	N (%)	Blade height (cm)	Blade width (cm)	Grass composition[z] (%)	Visual blade color (Munsell color chips) (Hue)	(Value)	(Chroma)
0	2.23d[y]	32.1c	0.51c	73.2c	6.99c	4.04a	5.56a
151	2.95c	49.8b	0.59b	90.2b	6.65c	3.81a	5.26a
454	3.75b	67.8a	0.77a	98.1a	7.51b	3.28b	4.34b
908	4.16a	69.0a	0.81a	99.3a	8.23a	3.00c	4.06b

[z]Composition of cover crop includes other species not mentioned (alfalfa, red clover and white clover) to equal 100% cover.

[y]Means in a column not followed by a common letter are significantly different by Duncan's Multiple Range Test ($P \leq 0.05$).

Apple Leaf N, Leaf Color, and Fruit Color

Leaf N content, leaf color and fruit color were markedly affected by different N rates (Table 3). Increasing N rates resulted in higher N concentrations and darker green color of leaves and peel color of fruit. Comparisons of leaf color of whole trees in the orchard from a distance of 8 to 10 m (field) with individual leaves in the laboratory (lab) were very similar indicating that quick visual estimates of leaf color with color charts (Figure 1) is possible to predict leaf N status of the tree. However, to obtain true color comparisons in the orchard, cloudy days should be avoided. The same leaf color chart with Munsell color chips was used for both orchard and laboratory determinations, but the color chips should be protected from fading due to light exposure when not in use. Over a six-year period, the best leaf N predictions (correlation values) between mid-terminal leaf N and leaf color estimates were with Munsell values (−0.796) and Munsell chroma (−0.833).

In a previous study (Raese and Williams, 1974), the relationships between leaf N and Munsell color chips (hue, value and chroma) were highly significant (0.815 to 0.937) for either spur leaves or mid-terminal leaves. In the final year of this study (Raese, 1978), leaf N concentrations and fruit color were significantly related to leaf color (Munsell color chips or reflectance) for all three leaf locations (mid-terminal, non-fruiting spur leaves or fruiting-spur leaves). However, highest correlations for leaf color and fruit yield were leaf color in fruiting-spur leaves (Raese, 1978). Fruit color was highly related to leaf N concentrations and leaf color when measured either subjectively by comparing with Munsell color chips or by instrument with the reflectance meter (Raese and Williams, 1974). Over the 6-year period of this study, fruit color was correlated to mid-shoot leaves (0.750) and to fruiting spur leaves (0.831).

Table 3

Effect of nitrogen (N) fertilizer rates on leaf nitrogen concentrations, harvest fruit color and leaf color of mid-terminal 'Golden Delicious' leaves over a six-year period

		Visual leaf color (Munsell color chips)								
Treatment	N	Hue		Value		Chroma		Reflectance (%)		Visual fruit color
N (g/tree)	(%)	Lab.	Field	Lab.	Field	Lab.	Field	Lab.	Field	(1–4)[y]
0	2.00c[z]	5.49c	5.28c	3.52a	4.13a	5.06a	6.14a	18.8a	NA	2.99a
151	2.00c	5.54c	5.18c	3.44a	3.85b	4.95a	5.66a	18.8a	NA	2.76a
454	2.14b	6.05b	6.39b	3.16b	3.20c	4.47b	4.82b	16.3b	NA	2.35b
908	2.40a	6.73a	7.00a	2.87c	2.66d	4.11c	4.05c	14.5c	NA	2.01c

[z]Means in a column not followed by a common letter are significantly different by Duncan's Multiple Range Test ($P \geq 0.05$).
[y]Estimated fruit color at harvest using the USDA Fruit Color Chart, 1929 (1 = green; 4 = light yellow).

Return Bloom, Yield, and Tree Growth

Biennial bearing for each N treatment was quite apparent over the six-year period, because the previous year's lowest yield was followed by the next year's highest yield and the previous year's highest yield was followed by the next year's lowest fruit yield (data not shown). Although not significant statistically due to annual variations, return bloom and cumulative fruit yield were 22.9% and 13.1%, respectively, higher for the 908g N treatment than the 151 g N treatment (Table 4). It is also interesting to note that the data of return bloom and yield were closely related.

Variation in yield from year to year was greatest for the 151g N treatment. This is because the highest total annual increases of + 21.9 boxes/tree and the highest total annual decreases of −27.5 boxes/tree resulted in a net difference of −5.6 boxes/tree (Table 4). The high rate of N had the highest positive net difference of + 6.3 boxes/tree. This could be interpreted as the treatment that tended to correct biennial bearing better than the other N rates. Others (Drake et al., 2002) reported highest yield of 'Golden Delicious' apples with the highest N treatment applied in August in spite of biennial bearing tendencies. However, N treatments alone should not be considered a reliable cure for biennial bearing of 'Golden Delicious' apple trees. Williams and Billingsley (1974) reported a low correlation between yield and N treatment with only slightly greater yield with high N fertilization of 'Golden Delicious.'

Neither fruit size nor trunk girth was appreciably affected by the different N rates of fertilizer (Table 4). Shoot extension was increased with the higher N treatments, but leaf size was only slightly larger on a fresh weight basis. The number of nodes on a shoot was greater for the higher N rates but internode length was not affected by N treatment.

Fruit Quality

Fruit color was markedly influenced by N treatments as determined by visual inspection with hand-held color charts or by two different color instruments, the Difference Meter or the Agtron (Table 5). In all measurements, the peel of the fruit was greener with each added increment of N treatment. Trees fertilized with the 151 g N/tree had the smallest fruit and the firmest fruit. Fruit from trees with the highest N treatment had the lowest values for firmness, SSC and TA and the lowest fruit quality. In other studies (Drake et al., 2002; Raese and Drake, 1997), lower N fertilizer treatments resulted in higher fruit quality of 'Fuji' and 'Golden Delicious' apples.

Early or mid-harvest timing resulted in greener, smaller fruit with greater firmness and acidity but lower SSC than fruit that was harvested 3 weeks later (Table 5). Fruit from the upper portion of the tree was less green in peel color, larger and had a higher SSC than fruit from the lower portion of the tree. In this study, fruit quality appeared to be more desirable when fruit was harvested from

Table 4
Effect of six years of nitrogen (N) fertilizer rates on return bloom, fruit yield, biennial bearing, fruit weight and tree growth of 'Golden Delicious' apple trees

Treatment N (g/tree)	Return bloom (%)	Cumulative yield (boxes/tree)	Biennial bearing variation (boxes/tree)[z]	(diff.)[y]	Fruit wt. (g)	Trunk girth (cm)	Shoot extension (cm)	Leaf wt. (g)	Shoot nodes (#)	Internode length (cm)
0	52.9	63.7	+8.9 vs −7.3	+ 1.6	207a[x]	109a	23.8b	16.0a	13.0b	2.08a
151	48.2	59.9	+21.9 vs −27.5	−5.6	198a	112a	25.4b	16.3a	12.0c	1.89a
454	56.5	67.5	+9.0 vs −10.3	−1.3	204a	115a	30.5a	17.1a	15.6a	2.05a
908	62.5	68.9	+15.7 vs −9.4	+6.3	208a	115a	32.8a	17.5a	16.1a	2.09a

[z]Total annual increase in yield (on the positive or negative side each year).
[Y]Net total difference (between total positives and negatives).
[x]Means in a column not followed by a common letter are significantly different by Duncan's Multiple Range Test (Pe $\leq$ 0.05).

Table 5
Effect of nitrogen (N) fertilizer rates, time of harvest and fruit location on the tree on fruit quality of 'Golden Delicious' apples

	Color			Size		Internal Quality		
Treatment N (g/tree)	Visual (1–6)[z]	Color Difference Meter	Agtron (E5-W)	Wt. (g)	Circum. (cm)	Firmness (N)	SSC (%)	Acidity (% malic)
				grams actual N/tree				
0	5.4a[y]	59.5a	49.0c	209a	23.1a	61.8a	13.1a	0.31a
151	5.1ab	49.6b	51.0bc	184b	22.4b	62.3a	12.7ab	0.29a
454	4.8b	39.5c	53.1b	203a	23.1a	60.5b	12.5bc	0.28a
908	4.1c	26.9d	58.5a	202a	23.2a	59.2c	12.0d	0.27a
				Time of harvest				
Mid-Sept.	4.4c	35.5c	55.6a	190b	22.5b	60.0a	12.4b	0.31a
Late-Sept.	4.8b	41.7b	53.8b	197b	22.8b	57.8b	12.4b	0.29ab
Mid-Oct.	5.4a	54.5a	49.9c	214a	23.5a	56.0c	13.1a	0.27b
				Fruit location on the tree				
Upper	5.0a	43.6a	50.4b	211a	23.3a	60.5a	13.6a	0.33a
Lower	4.5a	36.7b	54.4a	189b	22.5b	61.4a	13.0b	0.32a

[z]Fruit color visually estimated (1 = green; 6 = yellow).
[Y]Means in a column, within treatments, not followed by a common letter are significantly different by Duncan's Multiple Range Test ($P \leq 0.05$).

the lower N fertilized-trees, at approximately 145 d from full bloom and from upper portions of the tree. Others (Williams and Billingsley, 1974; Yamazaki et al., 1977), concluded that maximum production of high-quality 'Golden Delicious' fruit was obtained when leaf N was maintained between 1.9 and 2.1%; trees tend to become biennial bearing when leaf N is below 1.8% N even though producing high quality fruit. Results in this current study showed that highest fruit quality was obtained when leaf N was 2.0%, but yield was variable (Tables 3, 4, and 5).

Cold Hardiness and Related Causes

For all three seasons, fall, winter and spring, trees receiving no N or the lower rate of 151 g N/tree were associated with the greatest cold hardiness (Table 6). In each case, the highest rate of N resulted in trees having the most susceptibility to cold injury according to artificial freeze tests on 1- and 2-year-old wood. In an earlier report (Raese, 1997), cold hardiness during the fall months was greater in 'Anjou' (*Pyrus communis* L.) pear trees receiving the lower rate of N in either late winter or late summer versus higher N rates, but by mid-winter the higher rate of N in late winter produced trees with the highest cold

Table 6

Effect of nitrogen (N) fertilizer rates on fall, winter and spring cold hardiness (T_{50}) of twigs and shoots of 'Golden Delicious' apple trees and related causes for cold hardiness

Treatment (g actual N/tree)	Cold hard.[z] T_{50} (°C or °F)		Spur leaf[y] N (%)	Leaf color[x] reflectance (%)	Shoot extension[w] (cm)	Sap sorbitol (mg/ml)
			Fall cold hardiness			
0	−16.9c	(+1.6c)[v]	1.6c	37.8a	10.6c	0.58a
151	−17.2c	(+1.1c)	1.7c	37.2a	8.6d	0.42b
454	−15.4b	(+4.4b)	1.8b	30.2b	13.1b	0.31c
908	−13.8a	(+7.1a)	2.1a	24.5c	15.6a	0.34c
			Winter cold hardiness			
	Cold hard.[z] T_{50} (°C or °F)		Twig CO_2 (ppm/100g)	Twig dry mater (%)	2-yr. wood sap (g/100g)	Sap sorbitol (mg/ml)
0	−28.7b	(−19.7a)	50.5b	55.5ab	6.1a	1.8a
151	−28.4b	(−19.1a)	43.5b	54.3b	5.9a	1.8a
454	−26.1a	(−15.0b)	60.0a	55.7a	6.4a	1.7ab
908	−25.7a	(−14.3b)	64.5a	56.1a	6.3a	1.5b
			Spring cold hardiness			
	Cold hard.[z] T_{50} (°C or °F)		Spur leaf[y] N (%)	Bud stage[u] (1–8)	Shoot extension[w] (cm)	Sap sorbitol (mg/ml)
0	−17.0b	(+1.4b)	2.1c	6.3a	14.5c	0.23c
151	−16.8b	(+1.8b)	2.1c	6.2a	13.0d	0.39b
454	−16.0ab	(+3.3ab)	2.4b	6.4a	18.6b	0.93a
908	−14.5a	(+5.8a)	2.6a	6.5a	19.7a	0.33bc

[z]Cold hardiness in this study is measured by the often-used T_{50} described by (Levitt, 1956) which is the temperature where 50% of the plant tissue is damaged. The lowest temperature indicates the greatest cold hardiness.

[Y]Leaf nitrogen (N) concentrations from spur leaves collected in Sept., or Oct. for fall and May, or June for the spring samples over a six-year period.

[X]Leaf color measured by reflectance meter where low %R values = green and higher %R values represent shades of yellow.

[W]Current shoot growth measured during the latter part of the growing season (fall) and early shoot growth during May or June (spring).

[V]Means in a column, within seasonal hardiness, not followed by the same letter are significantly different by Duncan's multiple range test ($P \leq 0.05$).

[U]Bud stage visually estimated where 8 = full bloom.

hardiness. In 'Golden Delicious,' cold hardiness in the fall months for the four N treatments appeared to be closely related to autumn N content in the leaves. In an earlier report (Rogers and Batjer, 1953), N concentration in 'Delicious' apple leaves decreased rapidly after 140 days from full bloom as defoliation approached. In 15 of 18 trials on 'Anjou' pears, cold hardiness was increased with calcium nitrate [$Ca(NO_3)_2$] when evaluated against ammonium nitrate or urea, using comparable N rates (Raese, 1996). Calcium appeared to be a key factor as attested by $Ca(NO_3)_2$fertilizer or calcium chloride ($CaCl_2$) spray studies for cold hardiness (Raese, 1996) and apple quality (Raese and Drake, 2002). However, it is interesting to note that there is a dearth of information available concerning nutrition and cold hardiness of fruit trees.

In this experiment, cold hardiness in fall and spring appeared to be related to tree vigor such as increased concentration of N in spur leaves (Table 6). This tree vigor relationship was also apparent for fall leaf color, shoot extension in the fall and spring and to a slight extent to bud stage development. During the winter period, the concentration of CO_2 (respiration) was apparently curtailed more in low N-treated trees than in the higher N treatments. This indicates that perhaps the high N-treated trees were more active with less cold tolerance as shown by artificial freeze tests. Therefore, it would seem reasonable to assume that cold hardiness should also be related to dry matter and sap extracted in 2-year old wood as explained in more detailed studies later (Tables 7 and 8; Figure 2). However, only higher sap content extracted for the higher N- treated trees appeared to follow this pattern (Table 6).

In a previous study (Williams and Raese, 1974), it was determined that sorbitol and sucrose may be important reserves of storage carbohydrates in resting apple trees. When artificial freeze tests on apple trees were undertaken at controlled temperatures, it was also determined that cold hardiness was related to high levels of sorbitol in the sap and to high levels of sucrose and other sugars in 2-year old wood (Raese et al., 1978). In the present study, the low or no N-treated trees were associated with higher sorbitol in the sap and greater cold hardiness than the higher N-treated trees in the fall and winter months (Table 6). However, this trend was not apparent for the spring months, because highest sorbitol occurred with the 454 g N treatment.

'Delicious' Experiment

In an effort to provide additional credence for the possible explanations for the cause of cold hardiness of 'Golden Delicious' in response to the four N treatments, a seasonal account of cold hardiness of 'Delicious' trees and related causes are averaged over several seasons in an adjacent orchard. Cold hardiness of 'Delicious' as measured by T_{50} varied from $-1.1°C$ in June to $-30.8°C$ in January (Figure 2). It was closely related to percent dry matter of 2-year old wood and total sugars (fructose, glucose, sucrose and sorbitol). Both ambient

Table 7
Relationship between monthly average temperatures and/or cold hardiness (T_{50}) and various sugars in 2-year wood of 'Delicious' apple trees over a 10-year period, Orondo, WA

	Monthly average temperature	Monthly average cold hardiness (T_{50})
Variables vs		
Cold hardiness (T_{50})	0.982***	*NA*
Monthly average (Temp)	*NA*	0.923***
Sorbitol in sap (mg.ml)	−0.826***	0.854***
In 2-year wood		
Dry matter (%)	−0.772**	−0.892***
Sap (g/100g)	−0.103ns	−0.132ns
Fructose (mg/g)	−0.771**	−0.786**
Glucose (mg/g)	−0.796 ∗∗	−0.816***
Sucrose (mg/g)	−0.905 ∗∗∗	−0.898***
Sorbitol (mg/g)	−0.855 ∗∗∗	−0.854***
Total sugars[z] (mg/g)	−0.875***	−0.877***

[z]Total sugars = fructose, glucose, sucrose and sorbitol.
**Significant at 0.01, ***Significance at 0.001, ns = non significant, NA= not applicable.

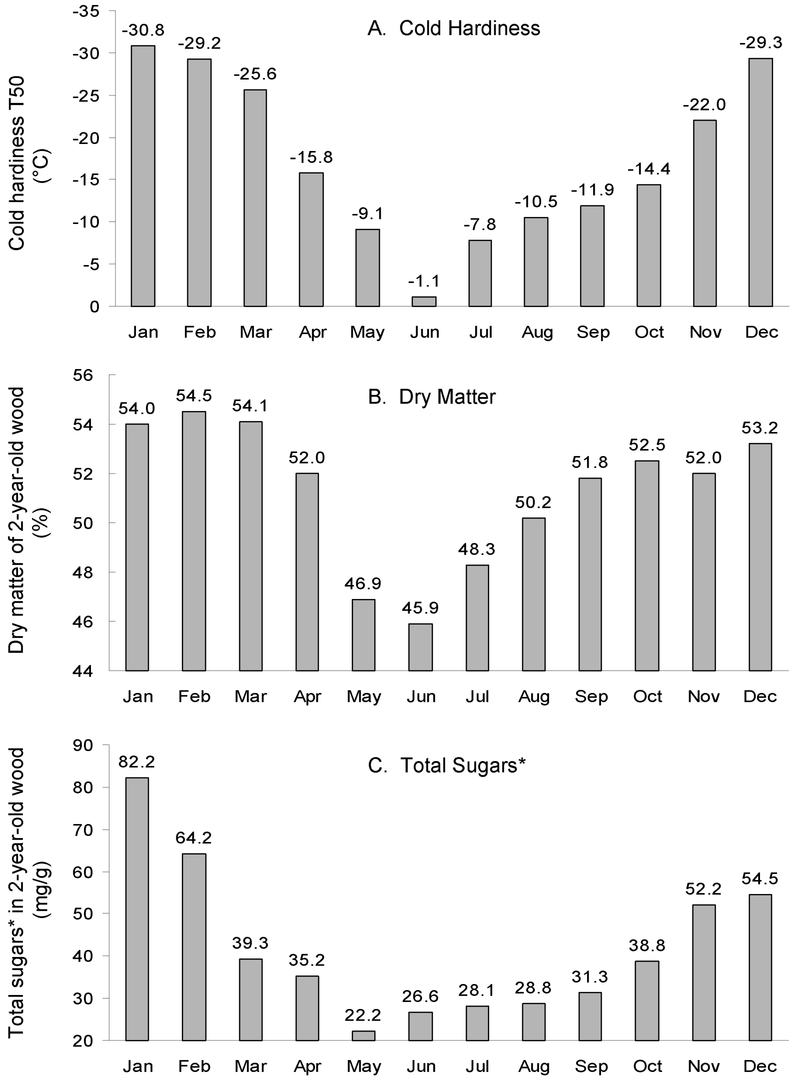
temperature and cold hardiness were closely related to each other and to percent dry matter, fructose, glucose, sucrose, sorbitol and total sugars in 2-year wood and sorbitol in sap (Table 7). However, they were not related to sap extracted from 2-year wood . It was reported earlier (Williams and Raese, 1974) that there was a close similarity between 'Golden Delicious' and 'Delicious' for the amount of sap extracted from wood and the amount of sorbitol in sap and wood during two dormant seasons.

It is common knowledge that temperature and cold hardiness of apple trees vary considerably over the four seasons of the year (Tables 7 and 8). Perhaps it is not so well known to what extent they vary with percent dry matter and sap extracted from 2-year old wood of 'Delicious' apple trees (Table 8). Cold hardiness appears to be greatest during months of lowest temperature, highest percent dry matter in 2-year old wood but moderate amounts of sap. Least cold hardiness occurs in months with higher temperatures, lowest percent dry matter, but also moderate amounts of sap in the wood. Highest amounts of sap occurred during late summer and early fall whereas lowest amounts of sap occurred in late winter and early spring months (Table 8). Therefore, the slightly more moderate levels of percent dry matter and sap associated with the low N and no N treatments (Table 6) may help explain the greater cold hardiness for those treatments in the winter months.

Table 8
Effect of different seasons and monthly average temperatures on cold hardiness, dry matter and sap extraction in 2-year wood of 'Delicious' apple trees over a 10-year period, determined at weekly intervals, Orondo, WA

Seasonal months	Temperature (°C)	Cold hardiness (T_{50})	Dry matter (%)	Sap extraction (g/100g)
Winter				
Jan	2.0	−30.8	54.0	6.7
Feb	2.9	−29.2	54.5	6.3
Mar	7.1	−25.6	54.1	5.8
Average	2.7	−28.5	54.2	6.3
Autumn				
Oct	11.5	−14.4	52.5	8.0
Nov	3.7	−22.0	52.5	5.7
Dec	0.7	−29.3	53.2	6.3
Average	5.3	−21.9	52.7	5.6
Spring				
Apr	11.3	−15.8	52.0	4.7
May	16.2	−9.1	46.9	5.7
Jun	20.6	−1.1	45.9	6.3
Average	16.0	−9.7	48.3	5.6
Summer				
Jul	24.7	−7.8	48.3	6.9
Aug	23.7	−10.5	50.2	7.3
Sep	18.5	−11.9	51.8	7.3
Average	22.3	−10.1	50.1	7.2

Of the carbohydrates tested in this study, sucrose had the highest negative correlation with seasonal temperatures and cold hardiness (Table 7). Sorbitol in wood and sap was also highly correlated to seasonal temperature and cold hardiness. In a relatively recent text, Nilsen and Orcutt (1996) contend that some plants appear to accumulate carbohydrates such as sucrose and the sugar alcohol, sorbitol, and others and that they may serve to stabilize membranes under the stress of chilling; this may help to maintain favorable ionic and osmotic balances. They also suggest that sucrose and certain other carbohydrates appear to be important osmotically in retaining cellular water under dehydration stress. They state that Ca^{2+} is the most effective divalent cation relative to changing membrane fluidity and that small amounts of Ca^{2+} are essential to membrane stability, but excessive amounts are detrimental. In most instances, either $CaCl_2$ sprays or $Ca(NO_3)_2$ fertilizer treatments enhanced the cold tolerance of 'Anjou' pear trees when compared with other non-calcium treatments (Raese, 1996).



*Sugars = Fructose, Glucose, Sucrose and Sorbitol

Figure 2. Influence of season on cold hardiness, dry matter and total sugars of 2-year-old wood from 'Delicious' apple trees.

CONCLUSIONS

In general, long-term high N fertilizer treatments resulted in higher return bloom and greater cumulative yield, but the lower N treatments produced higher fruit quality and greater cold hardiness in mature "Golden Delicious" apple trees. Soil pH and soil Ca levels were lowered while soil NO_3-N content was increased with the high N treatment. It appears that much of the higher rates of N was used to produce growth and N content of the cover crop, orchardgrass, as it was higher in N concentration than N in 'Golden Delicious' leaves. Leaf color determinations were doable with Munsell color charts in the orchard or laboratory and could be used to estimate leaf N concentrations or fruit color of 'Golden Delicious.' Nitrogen fertilization could not overcome the biennial bearing tendencies but the high N rate had a net positive difference between years. Fruit quality was more desirable for the lower rates of N treatment. Artificial freeze tests revealed that cold hardiness in fall, winter and spring was greatest with the lower rates of N and that in apple trees it could be related to tree vigor, dry matter of the wood and to sucrose and sorbitol content in the sap and wood. Perhaps the no-N trees performed well in all aspects of horticulture because these older trees had adequate N reserves stored in the tree system (previous personal communication, N. R. Benson, retired Soil Scientist, Washington State University).

REFERENCES

Batjer, L. P., J. M. Bloxom, G. Auvil, and N. R. Benson. 1966. Tree fruit nutrition in central Washington. *Proceedings Washington State Horticulture Association* 62:201–207.

Benson, N. R., R. M. Bullock, I. C. Chmelir, and E. S. Degman. 1957. Effects of levels of nitrogen and pruning on Starking and Golden Delicious apples. *Proceedings of American Society for Horticultural Science* 70:27–39.

Compton, D. C., W. C. Granville, D. Boynton, and E. S. Phillips. 1946. Color standards for McIntosh apple leaves. *Cornell University Agriculture Experiment Station Bulletin* 824:1–15.

Drake, S. R., J. T. Raese, and T. J. Smith. 2002. Time of nitrogen application and its influence on 'Golden Delicious' apple yield and fruit quality. *Journal of Plant Nutrition* 25:143–157.

Ketchie, D. O., R. D. Bartram, and C. H. Beeman. 1976. Evaluation of winter injury occurring during 1968–69 in Northcentral Washington. Bulletin. 826, Washington State University, Pullman, WA.

Ketchie, D. O., C. H. Beeman, and A. L. Ballard. 1972. Relationship of electrolytic conductance to cold injury and acclimation in fruit trees. *Journal of American Society for Horticultural Science* 97:403–406.

Levitt, J. 1956. The hardiness of plants. Academic Press, Inc., New York, pp. 1–278.

Lindner, R. C. and C. P. Harley. 1942. A rapid method for determination of nitrogen in plant tissue. *Science* 96: 565–566.

Munsell, A. H. 1971. A color notation: An illustrated system defining all colors and their relation by measured scales of hue, value and chroma. Munsell Color Co., Inc., Baltimore, MD, pp.1–67.

Nilsen, E. T. and D. M. Orcutt. 1996. The physiology of plants under stress. John Wiley and Sons, Inc., New York, pp. 1–689.

Raese, J. T. 1978. Estimating the nitrogen status of Golden Delicious trees by color. p. 409-410. In: A.R. Ferguson, R.L. Bieleski and I.B. Ferguson (eds). Proceedings 8th International colloq. on plant analysis. and fertilizer. problems. DSIR Information No. 134. Auckland, New Zea1and.

Raese J. T. 1995. Tree Fruit Nutrition II: soil pH and apple tree growth. *Good Fruit Grower* 46(5):42–44.

Raese, J. T. 1996. Calcium nutrition affects cold hardiness, yield and fruit disorders of apple and pear trees. *Journal of Plant Nutrition* 19:1131–1155.

Raese, J. T. 1997. Cold tolerance, yield and fruit quality of 'd'Anjou' pears influenced by nitrogen fertilizer rates and time of application. *Journal of Plant Nutrition* 20:1007–1025.

Raese, J. T. 2000. Relationship of fertilizers on soil pH and fruit disorders of apples and pears. *Communications in Soil Science and Plant Analysis* 31:2787–2792.

Raese, J. T. and S. R. Drake. 1997. Nitrogen fertilization and elemental composition affects fruit quality of 'Fuji' apples. *Journal of Plant Nutrition* 20:1797–1809.

Raese, J. T. and S. R. Drake. 2002. Calcium spray materials and fruit calcium concentrations influence apple quality. *Journal of American Pomological Society* 56:136–143.

Raese, J. T. and M. W. Williams. 1974. The relationship between fruit color of 'Golden Delicious' apples and nitrogen content and color of leaves. *Journal of American Society for Horticultural Science* 99:332–378.

Raese, J. T., M. W. Williams, and H. D. Billingsley. 1977. Sorbitol and other carbohydrates in dormant apple shoots as influenced by controlled temperatures. *Cryobiology* 14:373–378.

Raese, J. T., M. W. Williams, and H. D. Billingsley. 1978. Cold hardiness, sorbitol and sugar levels of apple shoots as influenced by controlled temperatures and season. *Journal of American Society for Horticultural Science* 103:796–801.

Rogers, B. L. and L. P. Batjer. 1953. Seasonal trend of several nutrient elements in Delicious apple leaves expressed on a per cent and unit area basis. *Proceedings of American Society for Horticultural Science* 61:1–5.

Shear, G. M. and F. Horsfall. 1948. Color as an index of nitrogen content of leaves of York and Stayman apples. *Proceedings of American Society for Horticultural Science* 52:57–60.

Washington State University. 1981. Critical temperatures for blossom buds-apples. Extension Bull. 0913, Pullman, WA.

Williams, M. W. and G. C. Martin. 1967. Quantitative gas chromatography of sugars and related polyhydroxy compounds in horticultural crops. *HortScience* 2:68–69.

Williams, M. W. and H. D. Billingsley. 1974. Effect of nitrogen fertilizer on yield, size and color of 'Golden Delicious' apples. *Journal of American Society for Horticultural Science* 99:144–145.

Williams, M. W. and J. T. Raese. 1974. Sorbitol in tracheal sap of apple as related to temperature. *Physiologia Plantarum* 30:46–52.

Yamazaki, T., T. Niizuma, I. Matsui, and T. Taguchi. 1977. Studies on leaf analysis in relation to its practical use for diagnosis in Golden Delicious apple orchards. *Akita Fruit Tree Experiment Station Bulletin No.* 9:25–73.